Chemogenomics

Hugo Kubinyi
Germany

E-Mail kubinyi@t-online.de
HomePage www.kubinyi.de

„Chemical Biology“
screening of chemical libraries in biological systems (e.g. whole cells), to detect new phenotypes.

„Chemical Genetics“
investigation of specific signalling pathways, e.g. by the design of orthogonal ligand-protein pairs

„Chemogenomics“
aims to discover active and/or selective ligands for biologically related targets in a systematic manner, i.e. library screening vs. target families (GPCRs, integrins, nuclear receptors, protein kinases, proteases, phosphatases, etc.).
Discovery of Monastrol, a Small Molecule Inhibitor of Mitotic Spindle Bipolarity

**In vitro Differentiation of Embryonic Stem Cells**

TWS 119 induces neuron formation from embryonic stem cells by modulation of glycogen synthase kinase 3β (GSK 3β)


Cardiogenol C, from a 100,000-member heterocycles library, induces cardiac muscle cell formation from embryonic stem cells


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**Dedifferentiation and Redifferentiation in Amphibia**

Newt

regenerates limbs, tail and eye lens

P. A. Tsonis, Molecular Interventions 4, 81-83 (2004)
Reversine Dedifferentiates Adult Murine Cells

Discovered in kinase inhibitor libraries, dedifferentiates adult murine myotube cells to mesenchymal progenitor cells


Chemical Genetics: Inhibitor-insensitive Kinases

Chemical Genetics - Orthogonal Ligand-Protein Pairs for the Study of Signalling Pathways

The Chemical Universe

$10^{40} - 10^{120}$ compounds with C, H, O, N, P, S, F, Cl, Br, I, and MW < 500

Chemogenomics: The Chemical Universe

..... tested against the Target Universe
Chemogenomics

**Principle:** screening of all possible compounds against all possible targets (chemical world vs. the target world)

**Real world:** screening of compound classes, enriched compound collections, targeted or focused libraries against classes of related proteins (target families)

**Target families:** GPCRs, integrins, nuclear receptors, tyrosine and serine/threonine protein kinases, metalloproteases, serine proteases, aspartyl proteases, etc.

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**Strategies in Chemogenomics**

- a gene family approach to parallel drug discovery
- screens
- scaffold morphing
- target hopping

P. R. Caron et al., Drug Disc. World Fall 2001, 57-62
Chemogenomics: Aspartyl Protease Inhibitors

Remikiren

Aliskiren

Nelfinavir

Saquinavir

Amprenavir

Chemogenomics: Aspartyl Protease Inhibitors

Remikiren

Aliskiren

Nelfinavir

Saquinavir

Amprenavir
Chemogenomics: Metalloprotease Inhibitors

<table>
<thead>
<tr>
<th></th>
<th>NEP 24.11</th>
<th>ACE</th>
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<tbody>
<tr>
<td>IC\textsubscript{50} values</td>
<td>1.1 nM</td>
<td>5.5 nM</td>
</tr>
<tr>
<td></td>
<td>11.5 nM</td>
<td>16 nM</td>
</tr>
<tr>
<td></td>
<td>2 820 nM</td>
<td>11.5 nM</td>
</tr>
</tbody>
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\textbf{Captopril}: IC\textsubscript{50} = 23 nM

\textbf{Other ACE inhibitors}:

\[ \text{Ki} \text{ [\textmu M]} \]
- MMP-1: 0.2
- MMP-2: > 10
- MMP-3: > 10

\[ \text{IC}\textsubscript{50} \text{ [\textmu M]} \]
- PDE4: > 1

G. Müller, Target family-directed masterkeys and chemogenomics, in H. Kubinyi and G. Müller, Chemogenomics in Drug Discovery, 2004, pp. 7-41

SAR of Metalloprotease Inhibitors

\[ K_i \text{ [\textmu M]} \]
- MMP-1: 1.0
- MMP-2: 0.01
- MMP-3: 0.5

\[ \text{IC}\textsubscript{50} \text{ [\textmu M]} \]
- PDE4: > 10
- PDE4: 0.001
- PDE4: 0.03
BREED
a program for the "mutation" of ligands (Vertex)

Design of Dual Zn\(^{++}\)/Cysteine Protease Inhibitors

IC\(_{50}\) MMP-1 = 3 nM  
IC\(_{50}\) Cat L = >1000 nM

IC\(_{50}\) MMP-1 = >1000 nM  
IC\(_{50}\) Cat L = 3 nM

IC\(_{50}\) MMP-1 = 25 nM  
IC\(_{50}\) Cat L = 15 nM


5-HT Receptor Subtypes (only GPCR’s)

J. Kelder, Organon, personal communication, 2001
Selectivity of 5-HT Receptor Ligands

\[
\begin{align*}
K_i (5-HT_3) &= 3.7 \text{ nM} \\
K_i (5-HT_4) &= > 1,000 \text{ nM}
\end{align*}
\]


cf. DF-1012 - orally active antitussive (guinea pig)


Selectivity of Uptake Inhibitors

<table>
<thead>
<tr>
<th>SNRI's</th>
<th>SSRI's</th>
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<tbody>
<tr>
<td>Talopram</td>
<td>Citalopram</td>
</tr>
<tr>
<td>Nisoxetine</td>
<td>Fluoxetine</td>
</tr>
<tr>
<td>0.0018</td>
<td>3 400</td>
</tr>
</tbody>
</table>

NA vs. 5-HT transporter IC\textsubscript{50} ratio (K. Gundertofte et al., in: Computer-Assisted Lead Finding and Optimization, HCA and VCH, 1997; pp. 445-459)
Different Modes of Action of Chemically Similar Molecules

promethazine (H₁ antagonist) chlorpromazine (dopamine antagonist) a, R = CH₃, imipramine b, R = H, desipramine (uptake blocker)

Many Ligands Bind to Several GPCRs

Olanzapine, a clozapine-like „atypical“ neuroleptic with a promiscuous binding pattern

a) F. P. Bymaster et al., Neuropsychopharmacology 14, 87-96 (1996)
Similarity of Various GPCR's (BLAST analysis)

light blue: aliphatic
green: polar
red: negative charge
cyan: aromatic
yellow: pro, gly

20, 115-123 (2001)

Different Modes of Action of Similar Molecules

Estrogen

Gestagen

Androgen

Anabolic
Steroid Analogs With Different Activities

- tamoxifene (antiestrogenic; breast cancer)
- cyproterone acetate (antiandrogenic; prostate cancer)
- mifepristone (antigestagenic; serendipitous discovery in search for a glucocorticoid antagonist)

Activities of Benzodiazepines

- diazepam (agonist)
- positive intrinsic activity at the GABA<sub>A</sub> receptor (tranquilizer)
- flumazenil (antagonist)
- no intrinsic activity at the GABA<sub>A</sub> receptor (antidot in intoxication)
- Ro 15-3505 (inverse agonist)
- negative intrinsic activity at the GABA<sub>A</sub> receptor (proconvulsant)
- tifluadom (opiate κ agonist, IC<sub>50</sub> = 12 nM)

The Concept of „Privileged Structures“

„these structures appear to contain common features which facilitate binding to various ... receptor surfaces, perhaps through binding elements different from those employed for binding of the natural ligands ....

... what is clear is that certain „privileged structures“ are capable of providing useful ligands for more than one receptor and that judicious modification of such structures could be a viable alternative in the search for new receptor agonists and antagonists.“

Privileged Structures

a) benzodiazepines (originally tranquilizers)

b) biphenyltetrazoles

c) di-Phe

d) spiropiperidines

CCK  opiate  NK-1

Ang I  Ang I  growth hormone
Privileged Structures

d) CNS-active phenylalkylamines

e) Aralkyl- and -aralkoxyamines with no CNS activity

Privileged Ring Systems (in 5120 drugs)

Most common side chains (of 5120 drugs)


Change of Therapeutic Focus

Mercurials
  antisyphilitic drugs - diuretics
Aspirin
  antiinflammatory - thrombozyte aggregation
  inhibition / cardioprotective - antitumour activity?
Sulfonamides
  antibacterials - diuretics, antihypertensives -
  antiglaucoma drugs - antidiabetics
Tricyclic drugs
  antihistaminics - neuroleptics - antidepressives
Verapamil
  coronary drug - antiarrhythmic - antihypertonic
Cyclosporin
  antimycotic - immunosuppressant
Diuretic, Antidiabetic and Anti-Glaucoma Agents from Antibacterial Sulfonamides

hydrochlorothiazide

carbutamide, $R = \text{NH}_2$
tolbutamide, $R = \text{CH}_3$
furosemide

glibenclamide
dorzolamide

Morphine and its Derivatives

morphine, $R_1 = R_2 = H$
heroin, $R_1 = R_2 = \text{acetyl}$ (opiates)
codeine, $R_1 = \text{Me}, R_2 = H$ (antitussive)
naloxone (morphine antagonist)
etorphine (2,000-10,000 times more active than morphine)
Distant Morphine Analogs

pethidine (meperidine)
first synthetic opiate
(derived from atropine)
atropine (anticonvulsant)
loperamide (obstipant)
haloperidol (neuroleptic)

Which Important Drug

started from an antiallergic lead, which was optimized to an antihypertensive drug but was finally clinically tested as an antianginal drug?

However, in a 10-day toleration study in Wales, an unusual side effect turned up ....

Zaprinast
unspecific PDE inhibitor; antiallergic, vasodilator.

Sildenafil (Viagra®), specific cGMP PDE5 inhibitor; male sexual dysfunction.
HIV-Protease Inhibitors from Anticoagulants

- **warfarin** (screening at Upjohn) 
  IC$_{50}$ = 30 µM
- **phenprocoumon** (similarity search at Upjohn) 
  IC$_{50}$ = 1 µM
- **U-96 988** (optimization at Upjohn) 
  IC$_{50}$ = 38 nM

- **screening at Parke/Davis**
  - **warfarin**
    - $K_i = 2.3$ µM
  - **phenprocoumon**
    - $K_i = 1.1$ µM
  - **U-96 988**
    - $K_i = 51$ nM

The SOSA Approach - Selective Optimization of Side Activities

„The most fruitful basis for the discovery of a new drug is to start with an old drug“

Sir James Black, Nobel Prize 1988

- **norfloxazin**, an antibiotic
- **flosequinan**, a mixed arterial and venous vasodilator

Anticholinergics, Antipsychotics, SSRIs, Tricyclics, etc.
"Selective Optimization of Side Activities"

\[ \beta \text{-blocker prototype} \rightarrow \text{cyclized analog} \rightarrow \text{levocromakalim, a hypotensive potassium channel opener} \]

norfloxazin, an antibiotic


"Selective Optimization of Side Activities"

\[ \text{minaprine, an antidepressant} \]

\[ \text{5-methyl isomer of minaprine} \]

\[ \text{tropane analog} \]

\[ \text{ortho-hydroxy substitution} \]

Selective Optimization of Side Activities

minaprine, an antidepressant

\[ K_i \text{ AChE} = 600 \, \mu M \]

desoxy, desmethyl-minaprine

\[ K_i \text{ AChE} = 13 \, \mu M \]

N-benzyl analog

\[ K_i \text{ AChE} = 120 \, nM \]

rigid analog

\[ K_i \text{ AChE} = 10 \, nM \]


3-Aminopyridazinines as 5-HT₃ Antagonists

minaprine, an antidepressant

\[ IC_{50} \text{ 5-HT}_3 = 425 \, nM \]

\[ IC_{50} \text{ 5-HT}_3 = 36 \, nM \]

\[ IC_{50} \text{ 5-HT}_3 = 370 \, nM \]

\[ IC_{50} \text{ 5-HT}_3 = 10 \, nM \]

Protein 3D Structure Similarity

Hugo Kubinyi, www.kubinyi.de

estrogen sulfotransferase (green) with cofactor PAP and substrate E2 (yellow)

uridylate kinase (blue) with cofactor ADP and substrate analog (red)


Protein 3D Structure Similarity

Hugo Kubinyi, www.kubinyi.de

Tie-2 TK VEGFR-3 IGF1R

LTA₄H ACE Thermolysin

Chromosome Translocation in CML

bcr-abl fusion protein, a hybrid with constitutionally enhanced tyrosine protein kinase activity

22-, philadelphia chromosome, present in 90+% of all cases of chronic myelogenous leukemia

abl = tyr protein kinase
bcr = ser/thr protein kinase

9+ chromosome 9
chromosome 22

Hugo Kubinyi, www.kubinyi.de

Source: www.cellsignal.com
Development of STI 571 (Imatinib, Glivec®)

Amides inhibit also tyrosine kinases, such as bcr-abl

R1 = Me (instead H) abolishes undesired PKC affinity

N-Me-piperazine increases solubility

Gleevec® (May 2001)

K_i ABL = 38 nM; K_i PGDFR = 50 nM (PDGFR = platelet-derived growth factor receptor); > 1000-fold selective vs. EGFR, c-src, PKA, PKC_α

Affinity Chromatography Using Immobilised Kinase Inhibitors

Coupled compounds: e.g. SB203580

SB203580, p38 MAP kinase inhibitor

Preparative Analysis of SB203580-bound Proteins

Protein spots excised
Trypsin digestion
Mass spectrometry analysis
Identification of several new protein kinase targets of the p38 inhibitor SB203580

Cyclin-CDK2 Complex in its Activated Form


ATP Binding Site Pockets of Protein Kinases

Key Interactions of ATP in the CDK2 Active Site


Binding Mode of a Kinase Inhibitor

Kinase Inhibitors in Human Therapy

- Gefitinib (EGFR; non-small-cell lung cancer; USA, 2003)
- Imatinib (bcr-abl, KIT and PDGFRB; CML and GIST; USA, 2001)
- Erlotinib (EGFR; non-small-cell lung cancer; USA, 2004)
- Fasudil (ROCK1; i.v., brain hemorrhage; Japan, 1995)
- Eupatilin (ERK1, ERK2 and CDKs; gastritis; Korea, 2003)

M. Vieth et al., Drug Discov. today 10, 839-846 (2005)

Kinase Inhibitors in Phase III Studies

- Su 11248 (FLT3, KIT, KDR and PDGFRB; renal cell cancer and gastrointestinal neoplasms)
- Vatalanib (KDR; colorectal, colonic and rectal neoplasms)
- Lapatinib (EGFR and ERBB2; metastatic breast cancer)
- CEP 1347 (MAPK8 and MAPK9; Parkinson’s disease)

M. Vieth et al., Drug Discov. today 10, 839-846 (2005)
Evolutionary Tree of Kinases
(red dots indicate 113 tested kinases)

TK = non-receptor tyrosine kinases
RTK = receptor tyrosine kinases
TKL = tyrosine kinase-like kinases
CK = casein kinase family
PKA = protein kinase A family
CAMK = calcium/calmodulin-dependent kinases
CDK = cyclin-dependent kinases
MAPK = mitogen-activated kinases
CLK = Cdk-like kinases

Selectivity of Kinase Inhibitors
(20 inhibitors tested vs. 113 kinases)

Design of a Highly Selective RSK1 and RSK2 Inhibitor

- Only 11 out of 491 related kinases have a non-conserved cysteine in position 436.
- Only 3 of these 11 kinases have a (small) threonine in the “gatekeeper” position 493.
- Irreversible reaction with Cys436 produces a highly specific RSK1 and RSK2 inhibitor.

<table>
<thead>
<tr>
<th>RSK2 inhibition, IC$_{50}$ in µM</th>
<th></th>
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<tbody>
<tr>
<td>wild type</td>
<td>0.015</td>
</tr>
<tr>
<td>Cys436Val</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Thr493Met</td>
<td>3.4</td>
</tr>
</tbody>
</table>

- Other kinases, with only one “filter”, are not inhibited; single point mutation of either one amino acid produces inhibitor-sensitive kinases (e.g. a Fyn Val285Cys mutant, a v-Src Val281Cys mutant, and a MSK1 Met498Thr mutant).


Privileged structures
- GPCRs
- Ion channels
- Kinases
- Phosphodiesterases
- Binding site similarity
- Natural product libraries etc.,

Wiley-VCH, 2004